Neuron, Vol. 37, 751–764, March 6, 2003, Copyright ©2003 by Cell Press

Neuronal or Glial Progeny: Regional Differences in Radial Glia Fate

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Summary

The precursor function of the ubiquitous glial cell type in the developing central nervous system (CNS), the radial glia, is largely unknown. Using Cre/loxP in vivo fate mapping studies, we found that radial glia generate virtually all cortical projection neurons but not the interneurons originating in the ventral telencephalon. In contrast to the cerebral cortex, few neurons in the basal ganglia originate from radial glia, and in vitro lineage analysis revealed intrinsic differences in the potential of radial glia from the dorsal and ventral telencephalon. This shows that the progeny of radial glia not only differs profoundly between brain regions but also includes the majority of neurons in some parts of the CNS.

Introduction

Radial glial cells, the sole and ubiquitous glial cells in the developing CNS of vertebrates, have recently gained new attention due to their crucial role in a variety of developmental processes (for recent review, see Campbell and Götz, 2002). In particular, the role of radial glial cells as precursors in the developing central nervous system (CNS) was only recently directly addressed in cell lineage and time-lapse video microscopy analyses and implied radial glia as neuronal and glial, maybe even multipotent precursor cells (Gray and Sanes, 1992; Malatesta et al., 2000; Miyata et al., 2001; Noctor et al., 2001; Tamamaki et al., 2001; for review, see Alvarez-Buylla et al., 2001).

To appreciate the proposed changes in the concept of radial glial cells, it is important to clarify the definition of radial glia and their distinction from a morphologically very similar cell type, the earlier neuroepithelial cells. Neuroepithelial cells and radial glia both have long radial processes extending from the cell soma in the ventricular zone to the pial surface (Figures 1A and 1B). In contrast to neuroepithelial cells, however, radial glial cells exhibit hallmarks of astrocytes, such as the content of glycogen granules (Choi, 1981), and the expression of a number of molecules, characteristic for astrocytes in the adult CNS (the glia-fibrillary acidic protein [GFAP], the astrocyte-specific glutamate transporter [GLAST], the brain lipid binding protein [BLBP], or Tenascin C [TNC]; Figure 1C; for review, see Campbell and Götz, 2002). These glial features appear around the onset of neurogenesis, at embryonic day (E) 13 in the mouse telencephalon, in a defined subset of precursor cells located in the ventricular zone (Hartfuss et al., 2001; Heins et al., 2002; Noctor et al., 2002) and distinguish radial glia from neuroepithelial cells, the earliest precursors in the CNS present already prior to neurogenesis (Figures 1C, 2A, and 2B). However, radial glial cells also share few molecular characteristics with earlier neuroepithelial cells, such as nestin and the antigens recognized by the monoclonal antibodies RC1 and RC2, of which the latter most likely recognizes a posttranslational modification of nestin (Chanas-Sacré et al., 2000, and references therein). Nestin and RC1/RC2 immunoreactivity are already present at E9 in the mouse telencephalon, i.e., in neuroepithelial cells, about 3–4 days prior to the onset of the astroglial markers GLAST and BLBP (Hartfuss et al., 2001, and references therein). The GLAST- and BLBP-positive radial glial cells then continue to express nestin until their morphological transformation into astrocytes when GLAST and BLBP is maintained, but nestin is downregulated (Figure 1C and Hartfuss et al., 2001). Taken together, the hallmark of radial glia is its astroglial properties that differentiate around the onset of neurogenesis.

Importantly, the differentiation of radial glia indicated by the expression of astroglial traits is accompanied by further cell biological changes, such as the loss of tight junctional coupling and the upregulation of several adhesion and extracellular matrix molecules, such as R-cadherin and TNC (Aaku-Saraste et al., 1997; Stoykova et al., 1997). These molecular changes might all contribute to the role of radial glia in supporting the migration of neurons along their radial processes, a process that also starts at the onset of neurogenesis (Hatten, 1999; Rakic, 1972).

Due to these differentiated astroglial properties and their later transformation into astrocytes (Schmechel and Rakic, 1979; Voigt, 1989), radial glial cells were for a long time seen mostly as support structures and as a source for other radial glia and later astrocytes, but recently, cell lineage analysis showed that radial glial cells of the cerebral cortex also generate neurons in vitro and in vivo (Malatesta et al., 2000; Miyata et al., 2001; Noctor et al., 2001; Tamamaki et al., 2001; Heins et al., 2002). However, the importance of this revised role of radial glia is not yet clear, as the extent of their contribution to neurogenesis and the identity of the neurons derived from radial glial cells are still unknown. Moreover, it is still open whether the neurogenic lineage of radial glia observed in the cerebral cortex is an exception or the rule in the developing CNS. To address these issues, we examined the progeny of radial glial cells in different CNS regions in vivo by recombinase-based

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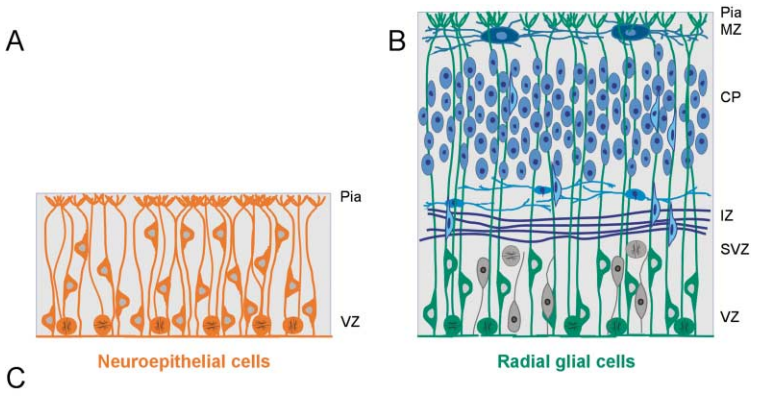

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Antigen/feature	Neuroepithelial cells	Radial Glia	Astrocytes
Nestin	+	+	- (reinduced in reactive astrocytes)
RC2	+	+	- (reinduced in reactive astrocytes)
RC1	+	+	+
GFAP	-	+ (absent in rodents)	+
GLAST	-	+	+
BLBP	-	+	+
TNC	-	+	+
Glycogen granules	-	+	+

Figure 1. Definition of Neuroepithelial Cells, Radial Glia, and Astrocytes

Panels (A) and (B) show schematic drawings of the cerebral cortex prior to neurogenesis (A) and at mid-neurogenesis (B). Note that neuroepithelial cells and radial glia have the same morphology but can be discriminated by several antigens, as depicted in (C). Also note that cells in the ventricular zone (VZ) undergo interkinetic nuclear migration and divide at the ventricular surface (A), while at later stages, a second proliferative layer, the subventricular zone (SVZ), develops where precursors undergo M phase at some distance from the ventricle (B). MZ, marginal zone; CP, cortical plate; IZ, intermediate zone.

fate mapping and in vitro by fluorescence-activated cell sorting (FACS).

Results

The hGFAP Promoter Directs CRE Recombinase to Radial Glia

To label the entire progeny of radial glia, we exploited a recombinase-based fate mapping technique that transforms transient gene expression into a permanent inheritable lineage marker (Dymecki and Tomasiewicz, 1998; Zinyk et al., 1998). This approach is based on the cell type-specific expression of Cre-recombinase that mediates the deletion of a stop cassette flanked by loxP sites (Figure 3A). Since we had previously observed that the human GFAP (hGFAP) promoter directs transgene expression not only to astrocytes in the adult CNS (Zhuo et al., 1997; Nolte et al., 2001) but also to radial glial cells during development (Malatesta et al., 2000), we carefully examined whether Cre under control of this promoter (Zhuo et al., 2001) is also targeted specifically to radial glia in the developing forebrain.

Cre immunoreactivity was not detectable in the neocortex and basal ganglia at E12, while many precursors were RC2 immunoreactive (Figures 2A and 2B). Correlated to the differentiation of glial marker expression (GLAST, BLBP), hGFAP-driven Cre immunoreactivity became prominent in the cortical ventricular zone (VZ) at E14 (Figures 2C–2H) and localized to a clear subset of precursors (detected by the monoclonal antibody Tec3 that recognizes the Ki67 antigen common to all dividing cells; Hartfuss et al., 2001). Notably, the majority of dividing Tec-3-immunoreactive precursors in the E14 cortex (67% $\pm$ 4%, n = 101, Figures 2C and 2C′) were Cre immunoreactive. In contrast to the labeling seen in the VZ, where radial glia somata reside, the precursors of the secondary proliferative layer (the subventricular zone [SVZ]) did not contain Cre (Figure 2D and see Supplemental Figure S1 available online at http://www.neuron.org/cgi/content/full/37/5/751/DC1). A similarly high proportion of Cre-immunoreactive precursors was also observed in the ganglionic eminence (GE) (62% $\pm$ 4%, n = 287). Thus, Cre is contained in about 2/3 of VZ precursors, reminiscent of the proportion of radial glial cells in the VZ (Hartfuss et al., 2001; Heins et al., 2002).

We used GLAST immunoreactivity to detect the majority of radial glial cells in the cortex and GE (Hartfuss et al., 2001). Almost all Cre-positive precursors in the VZ were GLAST positive (Cortex: 95% $\pm$ 2%, n = 174, Figures 2E and 2F; GE: 96% $\pm$ 1%, n = 262, Figure 2G), and the majority of GLAST-immunoreactive cells contained Cre immunoreactivity in the developing cortex (91% $\pm$ 4%, n = 181) and GE (94% $\pm$ 4%, n = 268, no difference between LGE and MGE, see also Figure 6B). Thus, the majority of radial glial cells contain Cre in this hGFAP-Cre mouse line, and no ectopic expression in other precursors or neurons could be detected (Figure 2H). Also in the adult CNS, Cre immunoreactivity was not present in neurons (Figure 2I), ependymal cells, oligodendrocytes (detected by CC1; Lu et al., 2002), or other glial precursors (detected by AN2, Diers-Fenger et al., 2001) but was detected only in GFAP-positive astroglial cells (Figures 2J and 2J′).

Region-Specific Differences in hGFAP-Cre-Mediated Recombination

Since the above analysis showed that Cre was targeted specifically to most radial glial cells in the developing forebrain, we crossed the hGFAP-Cre mice with different indicator strains (Lobe et al., 1999; Novak et al., 2000; Soriano, 1999) to visualize their progeny. In the R26R line (Soriano, 1999), the expression of Cre leads to a recombination event allowing *LacZ* expression from the ubiquitously active ROSA locus (Figure 3A). Therefore, the progeny of radial glial cells will express *LacZ* and appear blue in the histochemical X-Gal staining, while

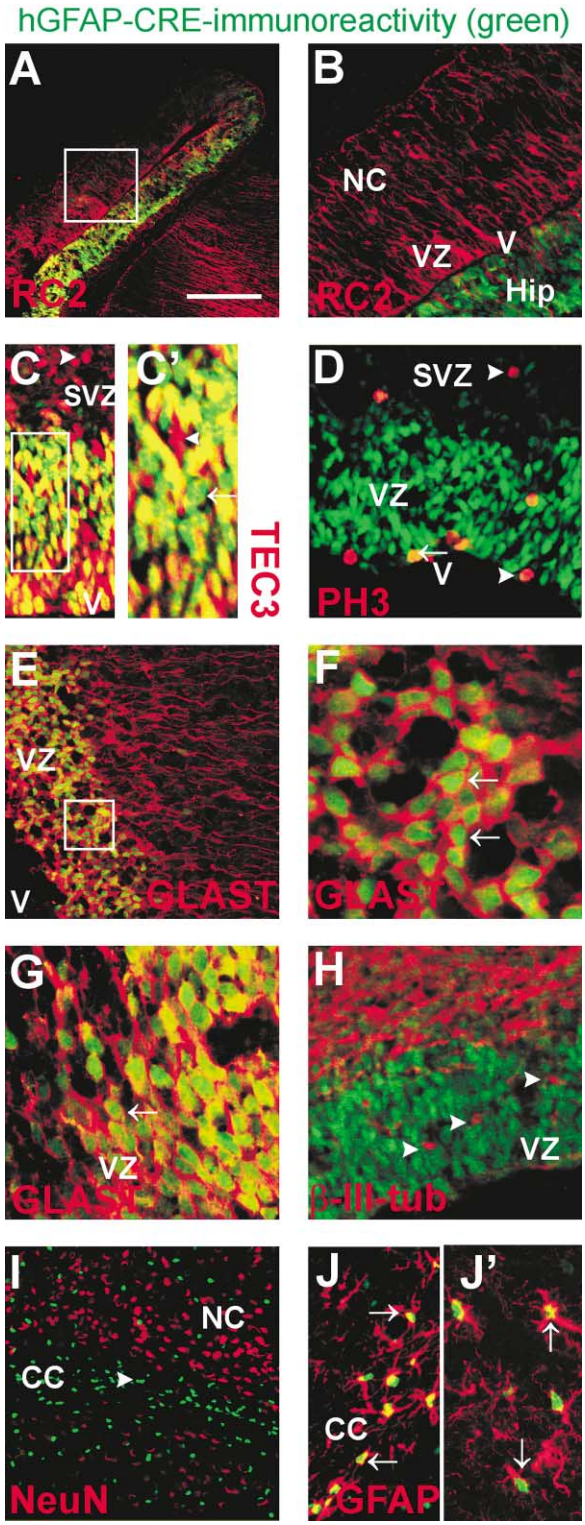

Figure 2. Expression of Cre from the Human GFAP Promoter

(A–J′) Fluorescent micrograph of frontal sections of the cerebral cortex (A–F and H–J), ganglionic eminence (G), or striatum (J′) from a hGFAP-Cre mouse at E12 (A and B), E14 (C–H), and P21 (I, J, and J′) stained for Cre (green) and the antigen shown in each panel (in red). Note the absence of immuno-detectable Cre at E12 in the neocortex, while it is detectable in parts of the hippocampal anlage (A and B). RC2 immunoreactivity is prominent throughout the CNS at this stage (A and B). Tec-3 labels dividing precursors (C), anti-PH3 cells in M phase (D), GLAST radial glia (E–G), β-tubulin-III (H), and NeuN (I) label neurons and GFAP astrocytes in the adult cortex (J) and striatum (J′). Note that in the hGFAP-Cre line, Cre immunoreactivity is localized in somata of GLAST-immunoreactive radial glia cells (E–G) but not in other precursors, such as those of the SVZ (C and D), or in neurons (H and I). Micrographs are single optical sections (1 μm). Arrows depict examples of double-labeled cells; arrowheads depict single-labeled cells. CC, corpus callosum; CP, cortical plate; Hip, hippocampal anlage; NC, neocortex; SVZ, subventricular zone; V, ventricle, VZ, ventricular zone. Scale bar, 400 μm (A); 100 μm (B and I); 65 μm (C); 80 μm (D and H); 150 μm (E); 25 μm (C′, F, and G); 50 μm (J); and 45 μm (J′).

the progeny of other precursors will remain unstained (Figures 3A–3C). At first sight, we noted prominent region-specific differences in the X-gal-positive cells in sections of postnatal (data not shown) and young adult (P21, Figure 3B) forebrain.

To examine whether partial failure of reporter activity in R26R mice could account for these region-specific differences, R26R mice were crossed with nestin-Cre mice (Graus-Porta et al., 2001). In these mice, homogeneous X-Gal labeling throughout the telencephalon was observed (data not shown), further supporting the specificity of the hGFAP-Cre localization in the radial glia subtype of precursors in contrast to the general recombination mediated in all precursors by Cre under control of the nestin enhancer. We also examined hGFAP-Cre-mediated recombination in two additional reporter lines, Z/AP and Z/EG (Lobe et al., 1999; Novak et al., 2000), which express a positive marker in cells that do not undergo Cre-mediated recombination (β-galactosidase, Figure 5A). In crosses of hGFAP-Cre and Z/AP or Z/EG reporter lines, the vast majority of neurons in the ventral telencephalon express β-galactosidase at early postnatal stages (Figures 5B and 5C) as well as in young adult animals (P21, Figures 5D and 5E), while those in the cortex express the marker indicating Cre-mediated recombination (Figure 5C). These results therefore show that most of the cells in the basal ganglia are not derived from Cre-expressing precursors in contrast to those in the cortex, implying lineage differences rather than problems with reporter activity as the cause for these region-specific differences in hGFAP-Cre-mediated recombination. We therefore carefully analyzed the identity of cells derived from the hGFAP-Cre-expressing radial glia in these regions.

Most Cortical Projection Neurons Derive from Radial Glia

In the cerebral cortex of hGFAP-Cre;R26R mice, many X-Gal-labeled or β-galactosidase-immunopositive (β-gal) cells were detected in the gray matter (GM) (Figures 3B–3J, see also Zhuo et al., 2001), and colocalization with NeuN revealed that most of them are neurons (Figures 3C–3E). Three-dimensional confocal analysis of double immunostaining of β-gal and NeuN confirmed the colocalization of these antigens within the same cell (Figures 3D′ and 3E′). While most of the NeuN-immunoreactive neurons in cortical layers 2–5 were β-galactosidase-positive (Figures 3C, 3D, and 3D′; Table 1), significantly fewer β-galactosidase-positive neurons were detected in layers 1 and 6b (Figures 3C, 3E, and 3E′; Table 1; Student's t test: $p < 0.005$). Note that these cells are mostly generated at E11/12 in mouse (Polleux

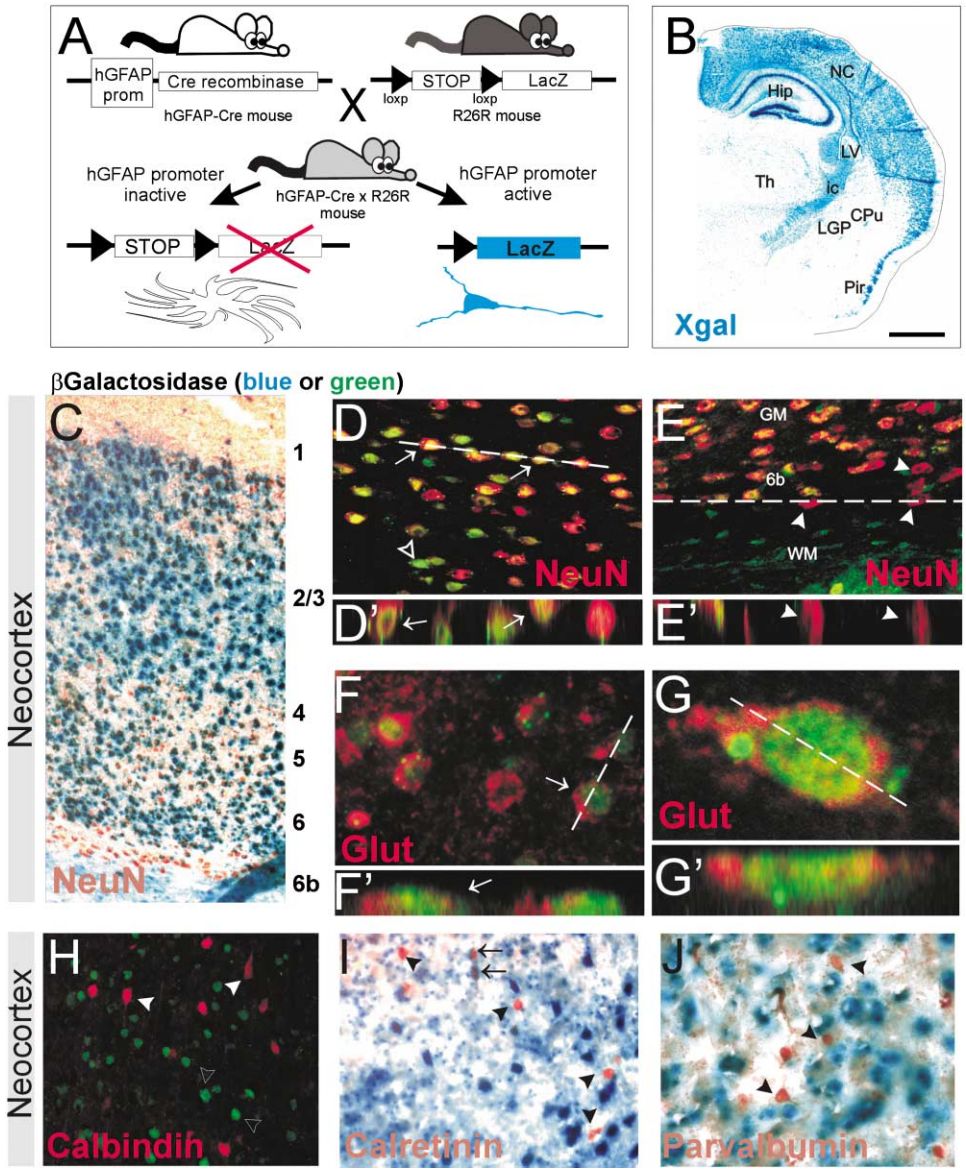

Figure 3. Cre-loxP-Mediated Fate Map of Radial Glia Progeny in the Cortex

(A) Schematic drawing of Cre-based fate map analysis. Crosses of the hGFAP-Cre mice (white in [A], Zhuo et al., 2001) and the reporter mice R26R (black in [A], Soriano, 1999) created offspring (gray in [A]) with radial glial cells that express the Cre-recombinase which excises the stop signal flanked by two loxP sites (black triangles). Radial glial cells and all their progeny will express *LacZ* (blue in [A]), while *LacZ* remains inactive in all the cells that do not derive form Cre-expressing progenitors (white in [A]).

(B–J) Detection of β-galactosidase in frontal sections of the hGFAP-Cre/R26R telencephalon (postnatal day 21) by X-Gal histochemical staining (blue; [B, C, I, and J]) or immunofluorescence (green; [D–H]). Note that most neurons (NeuN immunopositive) and pyramidal neurons (glutamate immunoreactive) located in the cortical Gray Matter (GM) are β-galactosidase positive (B–G), while β-galactosidase was rarely detected in Calbindin- (H), Calretinin- (I), or Parvalbumin-positive (J) interneurons. Double-labeled cells are indicated by arrows; single-labeled β-galactosidase-positive cells by empty arrowheads, and single marker-labeled cells by filled arrowheads. (D′)–(G′) are reconstructions of confocal sections in the Z axes at the level indicated by the white dashed line. CPu, caudate putamen; Hip, hippocampus; ic, inner capsule; LGP, lateral globus pallidus; LV, lateral ventricle; NC, neocortex; Pir, piriform cortex; Th, thalamus; GM, Gray Matter; WM, White Matter. Scale bar, 1 mm (B); 100 μm (C); 30 μm (D and E); 15 μm (F); 5 μm (G); and 50 μm (H, I, and J).

et al., 1997), i.e., prior to radial glia differentiation and the onset of Cre expression (Figures 2A and 2B). These data therefore confirm the close correlation between the detection of Cre immunoreactivity and functional recombination.

We also noted area-specific differences in the amount of radial glia-derived neurons, with about 90% of neurons expressing β-galactosidase in the isocortex and hippocampus, but only about 70% in the piriform cortex (Table 1, $p < 0.005$). Morphological and immunohistochemical analysis with glutamate-specific antisera showed that most pyramidal neurons that form the long-range projections of the cortex were β-galactosidase-positive (Figures 3F–3G′), while few interneurons identified by

Table 1. Identification of Neuronal Subtypes Derived from Radial Glia

Brain Region	Average (± SEM, n = Size of the Sample)			
Percent β-galactosidase-positive (radial glia-derived) neurons				
Cerebral cortex				
Isocortex				
Layer 2–6	88% (± 2%, n = 497)			
Layer 6b	31% (± 8%, n = 632)			
Layer 1	72% (± 4%, n = 276)			
Hippocampus				
Pyramidal cell layer	99% (± 2%, n = 104)			
Stratum radiatum	42% (± 7%, n = 56)			
Piriform cortex	66% (± 2%, n = 162)			
Basal ganglia				
Striatum	21% (± 4%, n = 1146)			
Globus pallidus	13% (± 4%, n = 194)			
Olfactory bulb				
Mitral layer	0% (n = 134)			
Granular layer	48% (± 2%, n = 129)			
Ant. olfactory nucl.	48% (± 11%, n = 169)			
Percent β-galactosidase-positive (radial glia-derived) interneurons				
	Parvalbumin	Calbindin	Calretinin	Er81
Cerebral cortex				
Isocortex	23% (± 2%, n = 1608)	24% (± 2%, n = 402)	38% (± 2%, n = 1384)	
Piriform cortex	31% (± 2%, n = 319)	53% (± 6%, n = 140)	52% (± 6%, n = 273)	
Striatum	79% (± 5%, n = 74)	3% (± 3%, n = 79)	16% (± 9%, n = 79)	69% (± 8%, n = 133)
Olfactory Bulb		49% (± 4%, n = 229)	55% (± 5%, n = 204)	86% (± 1%, n = 131)
Percent β-galactosidase-positive (radial glia-derived) projection neurons				
Basal ganglia				
Striatum	marker: DARP-32		12% (± 2%, n = 293)	
Globus pallidus	marker: Parvalbumin		2% (± 1%, n = 221)	

their GABA (data not shown), Calbindin, Calretinin, or Parvalbumin immunoreactivity contained β-galactosidase (Figures 3H–3J; Table 1). In contrast, a higher proportion of Calretinin- and Calbindin-positive interneurons containing β-galactosidase were detected in the olfactory bulb and the piriform cortex (Table 1). These data suggest that radial glial cells generate most projection neurons of the neocortex, but only some interneurons.

Few Neurons in the Basal Ganglia Derive from Radial Glia

Since most cortical interneurons originate in the GE (for review, see Marin and Rubenstein, 2001), we examined the radial glia progeny in this region. As described above, Cre immunoreactivity was also localized in the majority of radial glial precursors of the GE in similar numbers as in the cerebral cortex, suggesting that the low number of β-galactosidase-positive interneurons in the cortex is not due to a lack of Cre in the region from where they originate. Intriguingly and in pronounced contrast to the cortex, most neurons in the basal ganglia, the derivative of the GE, were β-galactosidase negative (Figure 4A; Table 1, significant difference to the cortex, $p < 0.005$). Most β-galactosidase-positive cells in this region were glial cells labeled with CC1, AN2, or BLBP (Figures 4B, 4C, and 4G). Unfortunately, we could not examine the lineage of astrocytes that should derive from radial glia, since the R26R-reporter line, as well as other reporter lines (Lobe et al., 1999), failed to express reporter activity in GFAP-positive astrocytes of the forebrain, despite their content of Cre (Figures 2J and 2J′) mediating recombination (Zhuo et al., 2001). No conclusions can therefore be drawn about astrocytes as progeny of radial glia. In contrast to the predominant glial progeny of radial glia in the GE, most β-galactosidase-positive cells in the cerebral cortex were neuronal (Figure 4G), suggesting pronounced differences in the progeny of radial glial cells from the cortex and the GE.

Precursors located in the ventral telencephalon are known to generate three main populations of neurons: the projection neurons of the striatum (caudate/putamen) that are GABAergic and DARPP32 positive (Ouimet et al., 1998); the GABAergic projection neurons of the globus pallidus, most of which contain Parvalbumin (Kita, 1994); and the interneurons that either remain in the basal ganglia or migrate to the cerebral cortex or the olfactory bulb (Marin and Rubenstein, 2001). The examination of the origin of the interneurons that leave the basal ganglia revealed considerable diversity in their lineage. While the majority of cortical interneurons were β-galactosidase negative, many periglomerular and granule cells in the olfactory bulb (labeled with Calbindin, Calretinin, and Er81 antisera) were β-galactosidase positive (Table 1, Figure 4E), suggesting that the interneurons destined for the cortex and those for the olfactory bulb originate from two distinct sets of precursors (Stenman et al., 2003). Similarly, our results revealed lineage diversity among the four classes of interneurons that remain in the basal ganglia: the Islet-positive cholinergic neurons, the GABAergic interneurons that contain

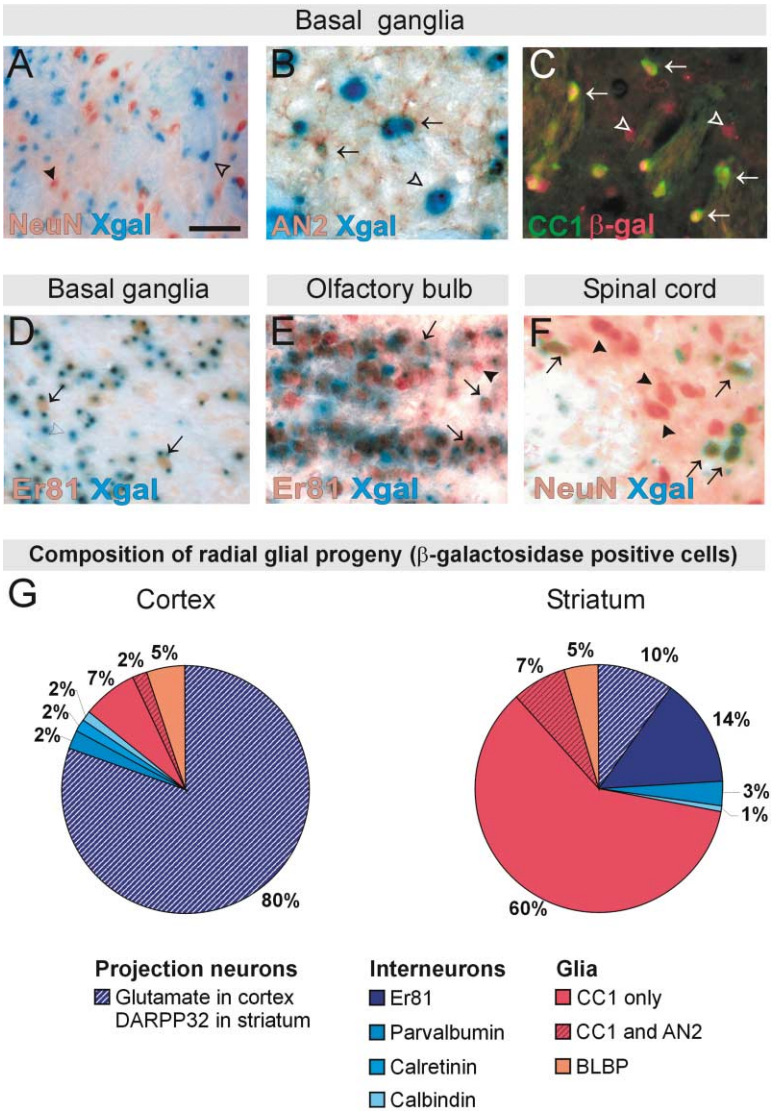

Figure 4. Cre-loxP-Mediated Fate Map of Radial Glia Progeny in the Basal Ganglia

(A)–(D) show that X-Gal (A, B, and D) or β-galactosidase-positive (C) cells originating from Cre-positive radial glia in the young adult (P21) basal ganglia are rarely neurons (A), but are often CC1-positive oligodendrocytes (C) or AN2-positive oligodendrocyte precursors (B). Notably, among the neurons, mostly Er81-positive interneurons in the striatum (D) and olfactory bulb (E) were β-galactosidase positive. (F) depicts β-galactosidase-positive neurons in the adult spinal cord. Arrows depict double-labeled cells, empty arrowheads single-labeled β-galactosidase-positive cells, and filled arrowheads single marker-labeled cells. Scale bar, 20 μm in (A), (C), and (D); 15 μm in (B) and (F); 17 μm in (E). The pies in (G) depict the composition of β-galactosidase-positive cells in the cerebral cortex and the striatum. Note that populations smaller than 1% (such as the Islet-positive cholinergic neurons) are not depicted. Colocalization was confirmed in double stainings. Note that most β-galactosidase-positive cells in the striatum are non-neuronal, in contrast to the cortex, where most are neurons.

either Parvalbumin or Calretinin or Calbindin/Somatostatin (Kawaguchi et al., 1995; Olsson et al., 1998; Marin et al., 2000), and the newly discovered Er81-positive interneurons (Stenman et al., 2003). While hardly any of the Islet- (5% ± 3%, n = 64), Calbindin- (3% ± 3%, n = 79), or Calretinin- (16% ± 9%, n = 79) positive interneurons contained β-galactosidase, most of the Er81- (69% ± 8%, n = 133) and Parvalbumin- (79% ± 5%, n = 74) positive interneurons were β-galactosidase positive (Table 1; Figure 4D). Since Er81-positive cells are more frequent than the Parvalbumin-positive interneurons, they account for the majority of the interneurons generated from radial glia in the striatum (Figure 4G).

In contrast to the diverse lineages of interneurons, projection neurons in the basal ganglia were largely not derived from radial glia. Very few DARPP32-positive projection neurons were β-galactosidase positive (12% ± 2%, n = 293), and they constitute only 10% of all cells derived from radial glia in the striatum (Figure 4G). Projection neurons of the striatum are further classified in neurons of the patch and matrix compartment that are discriminated by their birthdate (van der Kooy and Fishell, 1987). Since the earlier born neurons of the patch compartment constitute only about 10% of striatal projection neurons, we examined whether the DARPP32- and β-galactosidase-positive neurons were primarily in this compartment. However, when patch neurons were selectively labeled by DARPP32 immunostaining at late embryonic stages (E16), few of the DARPP32-positive cells contained β-galactosidase (4% ± 1%, n = 445). Comparable to the low number of striatal projection neurons derived from radial glia in the LGE, only very few (2% ± 1%, n = 221) projection neurons of the Globus Pallidus, the derivative of the MGE (Olsson et al., 1998), contained β-galactosidase. Taken together, the few neurons that are generated by radial glia in the GE contain few projection neurons, in contrast to the cerebral cortex, where radial glial cells generate the majority of projection neurons.

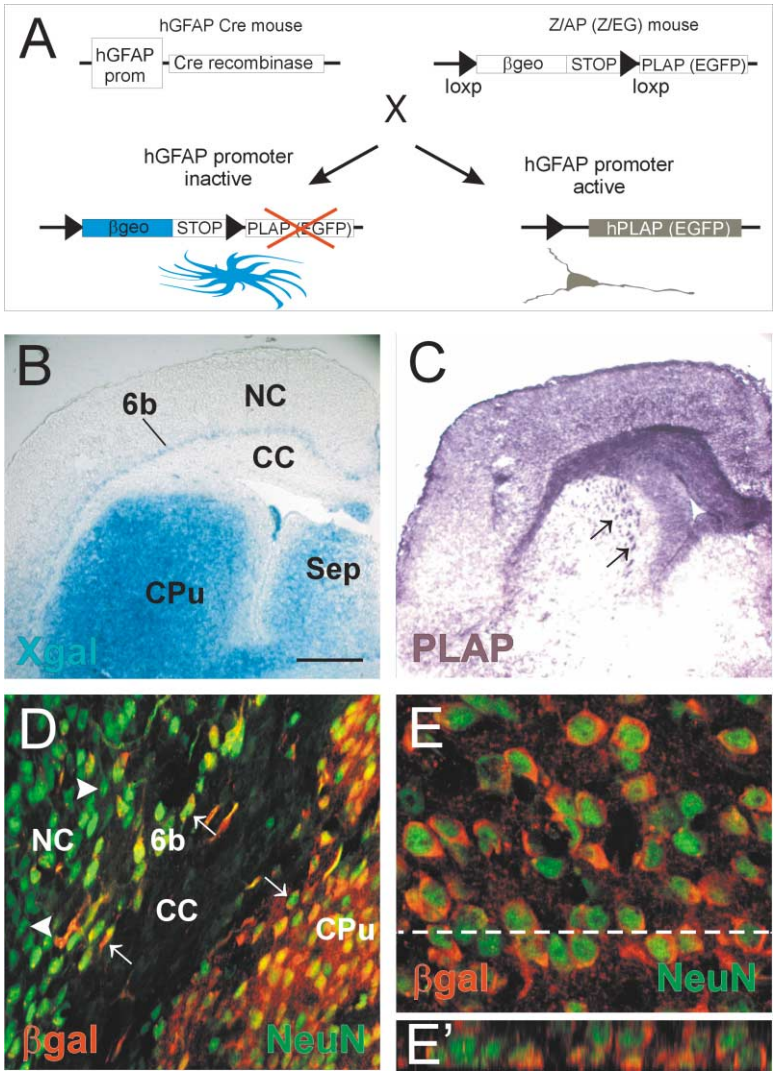

Figure 5. Neurons of the Ventral Telencephalon and Cortical Layer 6b Do Not Originate from Cre-Positive Radial Glia

(A) Cre-loxP-mediated fate map of radial glia using the hGFAP-Cre mouse line and the reporter lines Z/AP or Z/EG (Lobe et al., 1999; Novak et al., 2000). In the absence of Cre activity, cells express β-galactosidase from the β-geo gene (blue in [B]; red in [D] and [E]). In radial glia, the hGFAP promoter is active, and the Cre-recombinase mediates the excision of the stop cassette flanked by loxP sites and causes expression of human placental alkaline phosphatase in Z/AP (hPLAP, violet in [C]) or EGFP in Z/EG (data not shown). (B) and (C) show adjacent frontal sections of the telencephalon of hGFAP-Cre;Z/AP mice at postnatal day 1, stained histochemically for β-galactosidase (B) and PLAP (C). Arrows in (C) indicate fiber tracts in the Caudate/Putamen (CPu) that are PLAP positive. (D) and (E) show frontal sections of hGFAP-Cre;Z/EG mice at postnatal day 21, immunostained with antibodies against β-galactosidase (red) and the neuronal marker NeuN (green). (E′) shows a reconstruction of confocal sections in the Z axes at the level indicated by the white dashed line. Note that β-galactosidase labels mainly the neurons of the Caudate/Putamen and of the cortical layer 6b, showing that they are not derived from radial glia. CC, corpus callosum; CPu, Caudate/Putamen; NC, neocortex; Sep, septum. Scale bar, 1 mm (B and C); 30 μm (D); and 15 μm (E).

The Fate Differences between Radial Glia of the Cortex and the GE Are Cell Autonomous

The profound lineage differences observed between cortical and GE radial glia might be instructed by the local environment or rely on intrinsic lineage differences. To test these alternatives, we examined the progeny of individual radial glial cells, a clone, in vitro. Green fluorescent cells were isolated from three regions of the CNS (GE, cortex, spinal cord) by fluorescence-activated cell sorting (FACS) from a transgenic mouse line expressing EGFP under the hGFAP promoter (Nolte et al., 2001). As shown previously in a different hGFAP-GFP mouse line (Malatesta et al., 2000) and above for the hGFAP-Cre line, the hGFAP promoter directs EGFP to radial glial cells in all CNS regions analyzed (Figures 6A–6D). This was confirmed in sections and acutely dissociated cell preparations (Hartfuss et al., 2001), with most GFP-positive cells also GLAST or RC2 immunoreactive, but only 51% (n = 377) were BLBP positive (virtually no GFP-positive cells were GLAST-/BLBP positive but RC2 negative, consistent with the late appearance of this population, see Hartfuss et al., 2001). Hardly any differences were observed in transgene expression between MGE and LGE (Figure 6B), and a very similar composition of precursor cells as described above was detected when cells were analyzed shortly after isolation of green fluorescent cells by FACS.

To allow manipulation of the in vitro environment, we cultured the sorted cells on a feeder layer from either the same or a different brain region of rat embryos at corresponding ages. The use of the mouse neural-specific antibodies M2M6 allows visualization of the sorted cells from mouse CNS and their progeny on the immunonegative rat feeder layer (Figures 6E′, 6F′, and 6G′; see also Malatesta et al., 2000). Since fate change requires in most cases cell cycle progression (McConnell and Kaznowski, 1991), we examined exclusively the progeny generated from sorted cells undergoing at least one cell cycle in vitro, detected by BrdU incorporation (Figures 6E′, 6F′, and 6G′; see Experimental Procedures). As observed in vivo, also in vitro radial glia isolated from the GE and cultured in their normal environment generate very few neurons (Figure 6H) throughout development (E13–E17), with the highest proportion of neuronal

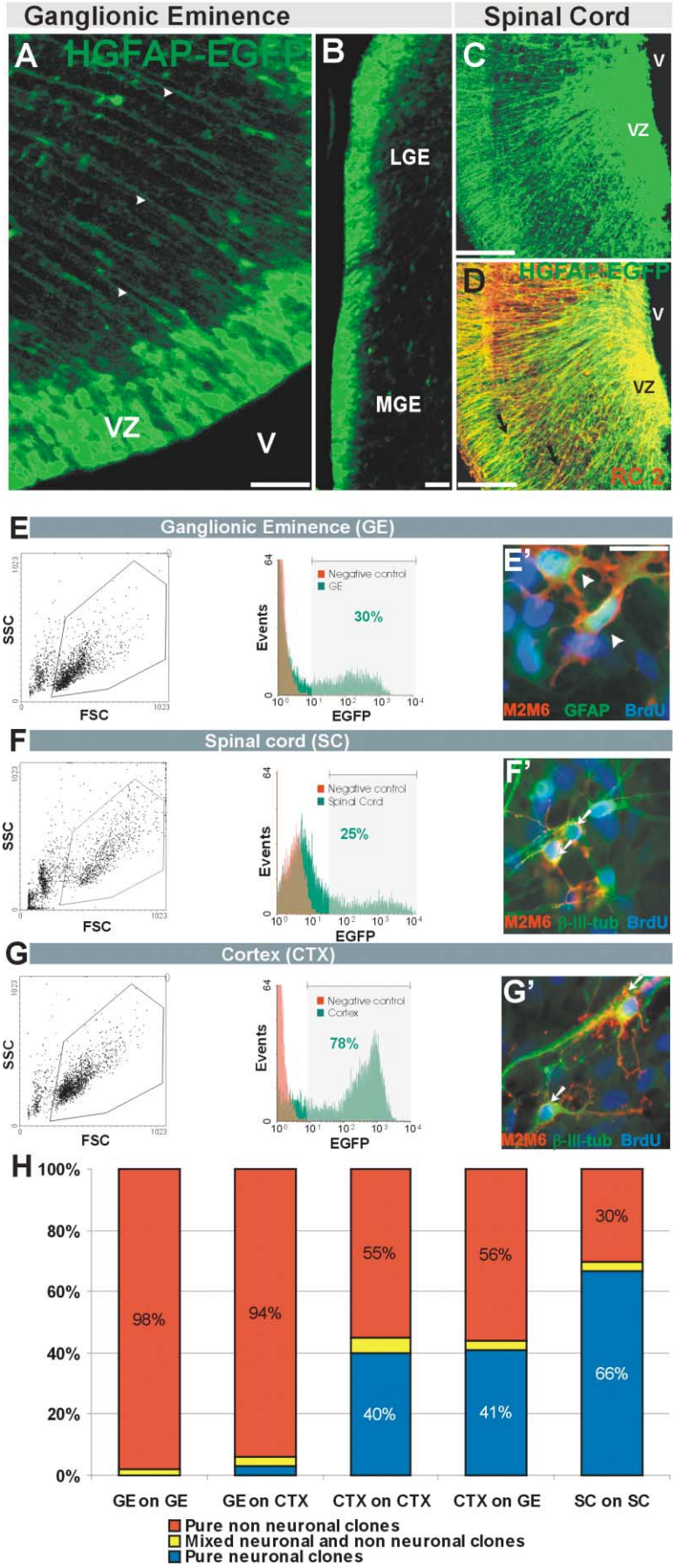

Figure 6. Clonal Analysis of Radial Glia Progeny In Vitro

(A–D) Confocal images ([A], single optical section; [B–D], maximum intensity projection) of frontal sections of the ganglionic eminence (A and B) and the spinal cord (C and D) of an E14 hGFAP-EGFP mouse embryo, stained with GFP antibody (green) and RC2 (red, [D]). (E)–(G) depict examples of fluorescent-activated cell sort (FACS) profiles of cells from the respective region of hGFAP-EGFP mice at E14 (E and G) and E12 (F). The left columns show the dot plots of cells in forward scatter (FSC) and side scatter (SSC) with a polygon indicating the gate selecting healthy cells. The histograms in the middle columns show the number of cells ("events," *y* axis) with a fluorescent intensity indicated on the *x* axis of wild-type controls (red) and hGFAP-EGFP transgenic littermates (green). The percentage of fluorescent cells in the sort gate (gray shading; light green) is indicated. (E′)–(G′) depict examples of clones derived from single sorted radial glial cells of the respective brain region. Sorted cells are plated at clonal density on a rat feeder layer, and their progeny is detected after 5–7 days in vitro as distinct cell clusters labeled by the mouse neural-specific monoclonal antibodies M2M6 (in red), the neuron-specific β-tubulin-III (green in [F′] and [G′]), or the astrocyte-specific GFAP (green in [E′]). The incorporation of the DNA-base analog BrdU (blue) indicates that cells divided in vitro. (E′) depicts a pure non-neuronal clone (both cells were GFAP positive but β-tubulin-III negative); (F′) and (G′) each show a pure neuronal clone (all cells β-tubulin-III positive) composed of two neurons. (H) The histogram represents the composition of the progeny of radial glial cells isolated by FACS after 5–7 days in vitro plated on different feeder layers. Note that hardly any BrdU-positive clones derived from the ganglionic eminence (GE) radial glia were neuronal, even when cultured on a cortical feeder layer (GE on CTX). In contrast, radial glia from the spinal cord (SC) and cortex (CTX) generated neurons in their normal (CTX on CTX; SC on SC) or a different environment (CTX on GE). Number of clones analyzed: GE on GE, 82; GE on CTX, 32; CTX on CTX, 189; CTX on GE, 85; SC on SC, 233. Scale bars, 60 μm (A); 100 μm (B); 120 μm (C and D); and 30 μm (E′–G′).

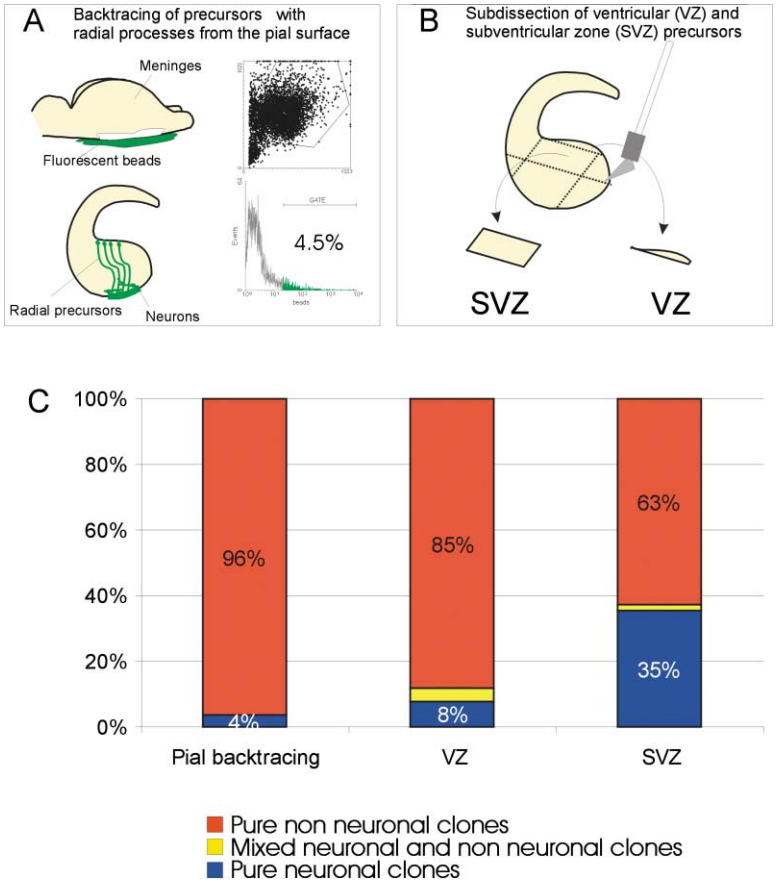

Figure 7. Clonal Analysis of Precursors from the Ventricular and Subventricular Zone In Vitro

(A) and (B) depict two ways to isolate ventricular zone (VZ) precursors independent of the hGFAP promoter activity. (A) Precursors with long radial processes were back labeled by green fluorescent beads applied to the surface of the GE after removal of the meninges. Cells that incorporated the green fluorescent beads were then isolated by FACS (profiles in right panels of [A]), and their clonal progeny was analyzed in vitro as described in Figure 6. (B) Schematic drawing illustrating the surgical separation of the VZ and SVZ regions in the GE in slices cut frontally with a tissue chopper. Cells were then dissociated and plated at clonal density on a rat feeder layer from GE as described in Figure 6. (C) The histogram depicts the composition of BrdU-positive clones generated by the precursors isolated as depicted in (A) and (B). Note that VZ precursors from the E14 GE enriched either by dissection or tracing from the pial surface generate few neurons, while precursors residing above the VZ mostly in the SVZ are more neurogenic. Number of clones analyzed: 27 (pial tracing), 138 (VZ), 72 (SVZ).

progeny at the earliest stage analyzed (E13: 21% ± 2% of all clones). Importantly, GE radial glia did not change their fate when plated on a cortex layer and still generated mostly non-neuronal cells (nestin- and/or GFAP-positive precursors and astrocytes, Figures 6E′ and 6H). In contrast, radial glia from the cortex or the spinal cord generated significantly more neuronal progeny ($p < 0.005$, Figures 6F–6H, see also Figure 4F for in vivo generation of neurons from spinal cord radial glia), even on a feeder layer from the GE (Figure 6H). Thus, the lineage differences of radial glial cells from different CNS regions persist in radial glia undergoing cell division in a different environment in vitro and hence seem to be cell autonomous.

Ventricular Zone Cells in the GE Are Not Neurogenic In Vitro

So far, our analysis in vivo and in vitro relied on the hGFAP promoter-mediated targeting of GLAST-positive cells. To examine the region-specific difference between the majority of VZ precursors by further independent approaches, we used first a previously established method to back-label precursors with long radial processes by application of fluorescent beads on the pial surface (see Malatesta et al., 2000). Since the radial processes of cells in the GE are curved ventrally, the fluorescent beads were applied by placing the brain on the beads-containing solution (Figure 7A). Consistent with our previous data obtained in the cortex, this incubation labels only a few percent of all cells in this region (4.5%, Figure 7A) that are mostly neurons (86% ± 1%) but that also comprise a small number of RC2-positive precursor cells (14% ± 2%, n = 179) as detected shortly after the sort. We assume that, in accordance to our previous data, the radial cells isolated by their morphology comprise not only GLAST-positive but also a small population of RC2-only-positive precursors (Malatesta et al., 2000; Figure 5). The progeny of proliferating precursors was then examined by the selective analysis of clones that had incorporated BrdU during the 5–7 days in vitro, thereby excluding the postmitotic neurons that were included among the sorted cells. Notably, hardly any neurons were generated from precursors of the GE that had been labeled from the pial surface (4%, Figure 7) in pronounced contrast to precursors labeled by the same procedure in the cortex (42%, Malatesta et al., 2000). These experiments therefore support the region-specific difference of VZ precursors from the cortex and the GE in their neurogenic potential.

To further confirm this region-specific difference and to elucidate the source of neurons in the GE, we took a second approach to separate VZ and SVZ precursors by surgical dissection. Telencephalic slices were cut at 300 μm thickness, and in each slice, a small stripe of the GE adjacent to the ventricle as well as a larger piece comprising the SVZ was isolated as depicted in Figure 7B. Indeed, we succeeded to largely deplete the SVZ fraction from VZ precursors, since only 11% of the SVZ fraction were RC2 immunoreactive (n = 121), an antigen

not present on SVZ precursors (Hartfuss et al., 2001). These mechanically separated fractions of GE precursors were then cultured at clonal density in the presence of BrdU on a rat feeder layer identical to the conditions of cells isolated by FACS. After 1 week in vitro, VZ cells enriched by dissection also generated hardly any neuronal clones, while many more were generated by the SVZ fraction (Figure 7C). There was no difference in the size of the clones generated by VZ (3.1 $\pm$ 0.2) or SVZ (3.6 $\pm$ 0.8) precursors, nor in the proportion of neurons contained in mixed clones (VZ, 24% neurons; SVZ, 22%). Thus, three independent means of labeling VZ cells in the GE show consistently that these precursors generate very few neurons and suggest that the majority of neurons in the GE arise from the SVZ precursor pool.

Discussion

Technical Considerations

Here we used a transgenic mouse line expressing Cre-recombinase under control of the hGFAP promoter (Zhuo et al., 2001) to monitor the progeny of radial glia by the inheritable recombination event mediated in different reporter lines. This approach depends critically on the faithful expression of Cre in radial glia during development. Indeed, Cre immunoreactivity is closely correlated to the appearance of radial glial cells after E12 in the cerebral cortex and GE and colocalized with the radial glial marker GLAST (Hartfuss et al., 2001), as observed in other hGFAP-transgenic lines (Malatesta et al., 2000). A quantitative colocalization analysis confirmed that Cre was contained in the majority of GLAST-positive precursors in both the cerebral cortex and GE. Since we have previously shown that GLAST is contained in both regions in the majority of radial glial cells (Hartfuss et al., 2001), we conclude that Cre is contained in the majority of radial glial cells in these regions. In contrast, Cre immunoreactivity was not detectable in neurons.

However, a low amount of Cre might not be detected by the antibody but still be sufficient to mediate recombination. For example, in the hGFAP-GFP mouse line (Zhuo et al., 1997), the FACS was able to detect a weak GFP signal in some neurons—supposedly derived from radial glia—which was not detected by immunostaining (Malatesta et al., 2000). However, in the hGFAP-Cre mice, this low expression, if it is there, seems not to be functionally relevant since there is a close correlation of immuno-detectable levels of Cre and functional recombination in vivo. Cre immunoreactivity was still very low in the neocortex at E12, the birthdate of neurons located in the layer 6b (Polleux et al., 1997). Consistently, in three reporter lines, most neurons in layer 6b were negative in R26R and β-galactosidase positive in Z/AP and Z/EG mice, indicating the lack of Cre-mediated recombination. In contrast, many more neurons that are generated after the onset of Cre immunoreactivity in the cortex (E13–E17) turned on the marker gene indicating Cre-mediated recombination (β-galactosidase in R26R, PLAP in Z/AP, and EGFP in Z/EG). Thus, Cre-mediated recombination in the cortex coincides with the time when Cre becomes detectable by immunostaining, a correlation also observed in other transgenic mice expressing Cre (Dragatsis and Zeitlin, 2000; Hirasawa et al., 2001). The converse problem of too low amounts of Cre to mediate recombination is more difficult to rule out, and it is important to note that Cre immunoreactivity appeared to be slightly weaker in the GE at early stages compared to the cortex.

Obviously, the best argument for the reliability of the Cre-based fate mapping is the similarity of the results obtained with three independent techniques. The region-specific difference in the neurogenic potential of radial glia isolated by FACS in vitro is an important control, since we can selectively analyze precursors that divided in vitro by adding BrdU to the culture medium. Therefore, any low expression of the transgene in neurons plays no role in these experiments, as they are excluded from the analysis. Moreover, the region-specific difference in the lineage of neurons in the basal ganglia and cerebral cortex was further confirmed by two approaches independent of the hGFAP transgene expression, by morphological back tracing of GE precursors via their radial processes from the pial surface as well as surgical enrichment of ventricular zone precursors. We are therefore confident that the recombination events mediated by Cre under control of the hGFAP promoter reflect the progeny of radial glial cells.

Regional Heterogeneity of the Radial Glial Lineage

Previous lineage tracing could follow the neurons generated by radial glia either only for a short time after their birth (Miyata et al., 2001; Noctor et al., 2001) or could only examine a few cells (Tamamaki et al., 2001), so the fate of most of the neuronal progeny of radial glia in the adult was not known. An important result obtained by the Cre-based fate map analysis of radial glia is therefore that they generate the majority of projection neurons in the neocortex. Noteworthy, cortical interneurons (labeled with Parvalbumin, Calbindin, and Calretinin) differ from projection neurons since they rarely contain the marker indicating Cre-mediated recombination, suggesting that the majority of them are not derived from radial glia, consistent with their origin in the GE (Marin and Rubenstein, 2001) where we found radial glia to be less neurogenic. The smaller fraction of cortical interneurons derived from radial glia seems obviously generated by a distinct lineage, potentially even within the cerebral cortex (see e.g., Letinic et al., 2002).

Even though Cre was contained in the same proportion of GLAST-positive radial glia of the GE, results obtained with three different reporter lines showed that the majority of neurons in the adult ventral telencephalon were not derived from Cre-positive radial glia. The glial cells that constituted the main derivative of GE radial glia were mostly oligodendroglia supposedly derived from the Olig2-positive radial glia in the MGE (see Supplemental Figure S1 at http://www.neuron.org/cgi/content/full/37/5/751/DC1). AN2 and BLBP were also contained in some of the glial progeny of radial glia, an intriguing finding in regard to the expression of both of these antigens in subsets of radial glia during development (Diers-Fenger et al., 2001; Hartfuss et al., 2001). However, no quantitative conclusions about glial subtype composition can be drawn, since most GFAP-positive astrocytes fail to report. This also explains why the

highest proportion of glial cells labeled is CC1-positive oligodendroglia. Interestingly, among the few GFAP-positive cells that have reporter activity in the R26R mice are the astrocytes in the subventricular/subependymal zone of the adult telencephalon that generate olfactory bulb interneurons throughout adulthood (Doetsch et al., 1999).

The few neurons generated by radial glia in the GE were particular subtypes of interneurons, further in line with the intriguing specificity of neuronal lineages originating from the GE (Marin and Rubenstein, 2001). In contrast to the Cholinergic/Islet-, Calretinin-, and Calbindin-positive interneurons that were not derived from radial glia, most of the Parvalbumin- and Er81-positive interneurons in the striatum seem to originate from hGFAP-Cre-positive precursors. Interestingly, the Cholinergic-, Calretinin-, and Calbindin-positive interneurons share common genetic pathways during their differentiation, depending on Dlx- and Mash1-expression, both of which are absent from the radial glial lineage (see below and Supplemental Figure S1 at http://www.neuron.org/cgi/content/full/37/5/751/DC1). In contrast, the Er81-positive interneurons of the striatum and the olfactory bulb seem to originate in the dorsal LGE (Stenman et al., 2003) where the transcription factor Gsh2 is expressed in radial glial cells (Supplemental Figure S1 at http://www.neuron.org/cgi/content/full/37/5/751/DC1). Since β-galactosidase-positive, Er-81-positive interneurons were already detected at perinatal stages in the olfactory bulb and the formation of the dorsal LGE and olfactory bulb is affected in Gsh-deficient mice (Corbin et al., 2000; Toresson et al., 2000; Toresson and Campbell, 2001; Yun et al., 2001), the Gsh2-positive radial glia in the dorsal LGE seem to contribute to olfactory bulb neurogenesis. Taken together, the few neurons generated by radial glial cells in the GE are highly specific subsets of interneurons, while radial glial cells in the cerebral cortex generate the vast majority of projection neurons.

Intrinsic Fate Determinants of Radial Glia Progeny

Despite the interesting nature of the neuronal subtypes generated by radial glial cells in the ventral and dorsal telencephalon, the most pronounced difference is in the amount of neurogenesis. The region-specific difference in the composition of β-galactosidase-positive cells in vivo (Figure 4G) is well consistent with our in vitro result that radial glia isolated from the GE also generate few neurons in contrast to those from the cortex. These experiments further showed that the fate of radial glia progeny is independent of the environment in vitro, suggesting that radial glia fate differs intrinsically between GE and cortex. Since the transcription factor Pax6 is expressed only in cortical but not GE radial glia (Götz et al., 1998) and the neurogenic radial glia is severely reduced in the Pax6 mutant cortex (Heins et al., 2002), Pax6 seems to act as cell-autonomous determinant of neurogenic radial glial fate in the developing telencephalon. Interestingly, Pax6 is also expressed in a subset of radial glia in the spinal cord (Ericson et al., 1997, and data not shown) and might also contribute to the neuronal lineage of the radial glia in this region. Moreover, Pax6 is correlated with adult neurogenesis in the rostral migratory stream (RMS; data not shown) and increases with enhanced neurogenesis after CNS lesions (Yamamoto et al., 2001). Thus, Pax6 could play a major role as neurogenic determinant in glial cells, not only during development, but also in the adult CNS.

Subventricular Zone Lineages

Since radial glia in the ventral telencephalon contribute little to the generation of neurons in the basal ganglia, different precursors must be responsible for their generation. Indeed, the GE contains a particularly prominent SVZ during embryogenesis, a region populated by precursors that undergo mitosis at some distance from the ventricular surface (Boulder Committee, 1970; Smart, 1976). In contrast to the cortex where the SVZ is very small, almost half of the progenitor pool of the GE is located in the SVZ (Smart, 1976, and Supplemental Figure S1 at http://www.neuron.org/cgi/content/full/37/5/751/DC1). Selective enrichment of VZ or SVZ precursors by surgical dissection confirmed that few of the VZ precursors in the GE generate neurons, while precursors located above the VZ generate neurons more frequently. Our in vitro data are further supported by previous genetic evidence implying the embryonic SVZ as the source of striatal neurons. The embryonic SVZ precursors express the transcription factors Dlx1, Dlx2, Mash1, and Ebf1 (Porteus et al., 1994; Eisenstat et al., 1999; Garel et al., 1999) that are absent from radial glia (for Mash1, see Supplemental Figure S1 at http://www.neuron.org/cgi/content/full/37/5/751/DC1; double-staining with pan-Dlx antiserum showed that only 16% of GLAST-positive cells are Dlx positive; Ebf1 is expressed at a considerable distance from the location of radial glia nuclei). Importantly, mice mutant for these transcription factors expressed mostly in the embryonic SVZ of the GE have defects in neurons of the striatum (Casarosa et al., 1999; Anderson et al., 1997; Eisenstat et al., 1999), supporting the role of Mash1 and Dlx genes in neurogenesis from SVZ precursors. Lineage tracing in the GE revealed clones containing neurons and radial glial cells (Reid and Walsh, 2002) that derive either from a radial glia cell generating a neuron and a radial glia (as observed in the cortex by Noctor et al., 2001) or from an earlier neuroepithelial precursor giving rise to a radial glia and neurogenic precursors, e.g., located in the SVZ. Indeed, neuroepithelial cells generating both SVZ and radial glia with mostly the former continuing to generate neurons is a scenario that would unify all presently available data. Surprisingly few clones containing neurons and radial glia have been described in the cortex, where radial glia is the main source of neurons. This is best explained by the observations of Miyata et al. (2001) that radial glial cells transform into the postmitotic neurons.

In contrast to the embryonic SVZ, the postnatal subependymal/subventricular zone in the GE seems to be derived from radial glia because it becomes β-galactosidase positive prior to the endogenous expression of GFAP in astrocytes of this region. The transformation of radial glia into precursors of the postnatal SVZ has further been observed in recent morphological DiI tracing analysis (Alves et al., 2002). These results therefore suggest some important differences between the postnatal, predominantly gliogenic (Levison and Goldman,

1993; Staugaitis et al., 2001) and the embryonic, apparently neurogenic SVZ. The key question that remains to be answered, however, is how the precursors of only a small region of the SVZ can maintain their neurogenic potential throughout adulthood (Doetsch et al., 1999). An intriguing possibility is that radial glial cells of the dorsal LGE that appear to generate interneurons of the olfactory bulb may continue to do so as astrocytes in the adult subventricular zone at later stages.

Final Remarks

The region-specific difference in the lineage of radial glia from the cortex and GE highlights the importance not to generalize the lineage of radial glia across species or CNS regions. Indeed, a hallmark of the developing primate cortex is its huge SVZ during neurogenesis (Smart et al., 2002), and hence the source of neurons in primate cortex has to be directly analyzed in this system. Even within the same species, the mouse, our data show that radial glial cells—as astrocytes at later stages (Denis-Donini et al., 1984; Doetsch et al., 1999; Wagner et al., 1999)—differ profoundly between brain regions. These region-specific differences are highly relevant for adult neurogenesis and the attempts to reconstitute specific types of degenerating neurons. Astroglial cells not only promote the number of neurons generated by adult neural precursors in vitro (Song et al., 2002) but also instruct specific neuronal phenotypes depending on their region of origin (Denis-Donini et al., 1984; Wagner et al., 1999; Skogh et al., 2001). In addition, in those regions where radial glia generate neurons, they seem to be specified to generate a particular type of neuron, e.g., glutamatergic pyramidal neurons in the cerebral cortex. Finally, glial cells with the properties of stem cells are restricted to few regions in the adult CNS, highlighting the importance of patterning mechanisms that endow these but not astrocytes in adjacent regions with neurogenic properties. The region-specific neurogenesis of glial cells thus holds the key to successful region-specific neuronal replacement.

Experimental Procedures

Animals

The hGFAP-CRE mouse line (Zhuo et al., 2001) has been maintained in SV129, the two reporter lines R26R (Soriano, 1999) and Z/EG (Novak et al., 2000) in the C57BL6/J, and the Z/AP line (Lobe et al., 1999) and hGFAP-EGFP (Nolte et al., 2001) in the FVB/N background. We noted no major differences in the reporter activity in the F1 crosses on these different mixed backgrounds (Hebert and McConnell, 2000). Animals have been handled in compliance with European and German law.

Immunostainings

Immunostainings were performed as described previously (Hartfuss et al., 2001). Brains were fixed by immersion (embryonic brains, 2% Paraformaldehyde at 4°C for 6 hr) or by perfusion (adult brains). The following antibodies were used: rabbit polyclonal (pAb) anti-Calretinin and anti-Calbindin (1:500, Swant), rabbit pAb anti-Cre (1:5000, Covance Research Product), rabbit pAb anti-DARPP-32 (1:100, Chemicon), rabbit pAb anti-Er81 (1:5000, kindly provided by S. Morton, S. Arber, and T. Jessell), rabbit pAb anti-β-galactosidase (1:3000, Cappel), rabbit pAb anti-Parvalbumin (1:500, Swant), rabbit pAb anti-PH3 (1:200, Biomol), guinea pig pAb anti-GLAST (1:8000, Chemicon), mouse monoclonal (mAb) anti-Calbindin (IgG1, 1:200, Sigma), mAb anti-CC1 (IgG2b, 1:200, Oncogene), mAb anti-GFAP (IgG1, 1:200, Sigma), mAb anti-Islet1 (1:2, T. Jessel, Dev. Hybridoma Bank), mAb anti-nestin (IgG1 1:4, Dev. Hybridoma Bank), mAb anti-NeuN (IgG1, 1:50 Chemicon), mAb anti-RC2 (IgM, 1:500, P. Leprince), mAb anti-β-tubulin-III (IgG2b; 1:100, Sigma), rat mAb anti-Ki67 (Tec3 1:50; Dianova), rat mAb anti-AN2/NG2 (1:25, kindly provided by J. Trotter, University of Mainz, Germany; see Hartfuss et al., 2001). For detection, we used fluorescent secondary antibodies (Europath or TSA; NEN) or 3, 3-diaminobenzidine (DAB) histochemical detection (ABC Elite and DAB kits, Vector Laboratories) combined with standard X-Gal histochemical staining, analyzed by epifluorescence (Zeiss Axiophot) or confocal laser scanning microscopy (CLSM, Leica TCS NT).

Fluorescent Activated Cell Sorting

Cortex (Ctx), ganglionic eminence (GE), and spinal cord (SC) were dissected from hGFAP-EGFP mice (Nolte et al., 2001) at E12 (SC) and E14 (Ctx, GE). For the labeling of precursors with radial morphology from the pial surface, the meninges were removed from telencephali and placed into a solution of green fluorescent beads (Lumafluor 50 nm; ventral side down for GE labeling, see Figure 7A and Malatesta et al., 2000). A further incubation of 15 min before dissection allowed sufficient time for transport of the fluorescent beads, as demonstrated previously (Malatesta et al., 2000). Fluorescent cells were isolated using a FACSort or FACSVantage (BD). By reanalysis, a purity of 95% was confirmed. To separate ventricular from subventricular zone cells, 300 μm thick slices were cut from mouse E14 telencephalon using a McIlwain tissue chopper, and the respective regions were then dissected in each slice as depicted in Figure 7B. Cells were dissociated as described previously (Malatesta et al., 2000) and plated on Poly-D-lysine (PDL) coated coverslips, in the presence of a feeder layer derived either from the same or from different CNS region of rat embryos at the corresponding developmental stage. Cultures were maintained in chemically defined medium (SATO, see Malatesta et al., 2000) in the presence of the DNA base analog 5-bromo-2′-deoxyuridine (BrdU) for 5–7 days in vitro.

Acknowledgments

We are very grateful to A. Messing for the generous gift of the hGFAP-Cre mice; to P. Soriano for the R26R mice; to C. Lobe for the Z/AP and Z/EG mice; and to S. Arber, K. Campbell, T. Jessel, P. Leprince, S. Morton, and J. Trotter for the kind gift of antibodies. We would like to thank K. Campbell particularly for all his excellent suggestions and discussion throughout the progress of this work. We are also very grateful to Y. Barde, B. Grothe, F. Guillemot, H. Thoenen, and A. Kriegstein for very useful discussions and comments on the manuscript; L. Lindemann and C. Parras for great help with technical problems; N. Haubst for providing data on the SVZ; and M. Öcalan and P. Ghahraman for excellent technical expertise. The antibodies against Nestin and Islet1 have been obtained from Developmental Studies Hybridoma Bank. This work was supported by the Human Frontiers Science Program, European Union, Deutsche Forschungsgemeinschaft, and the Max-Planck Society.

Received: August 8, 2002
Revised: February 5, 2003

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